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# Mastitis in small ruminants.<sup>☆</sup>

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#### Abstract

This manuscript reviews the most recent knowledge about small ruminant mastitis, pointing out the etiological, epidemiological and control aspects of mastitis. The prevalence of subclinical mastitis in small ruminants averages 5–30%, but the annual incidence of clinical mastitis is generally lower than 5%. *Staphylococcus* spp., are the most prevalent pathogens responsible for intramammary infection in small ruminants. Mastitis caused by *Staphylococcus aureus* should be eliminated because of the severity of the clinical symptoms and also because of the risk of contamination of milk products by thermostable toxins. The public health impact of other pathogens causing mastitis is also emphasized in this review, and the efficacy of diagnostic tools is discussed, especially diagnostic bacteriological tests and determination of milk somatic cell counts (MSCC). Several mastitis control strategies are discussed, such as milking procedures, teat dip disinfection and selective dry-off therapy.

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### 1. Introduction

Because important differences exist among dairy ruminants, the approach to mastitis control in goats and sheep should be carefully made with a specific point of view, and not by generalizing results obtained from research on mastitis in dairy cows. Current knowledge of mastitis in small ruminants has been recently reviewed by authors such as Bergonier et al. (2003). More specifically, the role of intramammary pathogens in mastitis

in goats has been reviewed by Contreras et al. (2003), and Bergonier and Berthelot (2003) have reviewed the epidemiology and control of mastitis in sheep. Other studies include those of Paape et al. (2001), who explored the feasibility of indirectly diagnosing mastitis in small ruminants by using MSCC, and of Gonzalo (2004), who recently discussed the analytical, health, productive and technological aspects of performing MSCC in sheep and goat milk.

# 2. Epidemiological aspects of small ruminant mastitis

The annual incidence of clinical mastitis in small ruminants is generally lower than 5%, but this incidence can increase sporadically. The prevalence of

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subclinical mastitis has been estimated at 5–30% or even higher (Bergonier and Berthelot, 2003; Contreras et al., 2003), but there are only limited data about incidence of intramammary infection (IMI) of goat and sheep in the literature.

Several pathogens can cause mastitis but *Staphylococcus* spp. are the most frequently diagnosed causal microorganisms of IMI in goats and sheep (Tables 1 and 2). Other pathogens such as *Streptococcus* spp., *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Mannheimia haemolytica*, *Corynebacteria* and fungi can produce IMI in small ruminants, but occurrence rates are lower. In addition, severe cases of mastitis related to incorrect preventative strategies have been attributed to the pathogens *Aspergillus fumigatus*, *Serratia marcescens*, *P. aeruginosa* or *Burkholdelia cepacia* (Las Heras et al., 1999b; Berriatua et al., 2001; Bergonier and Berthelot, 2003; Contreras et al., 2003; Gonzalo et al., 2004b).

Lentiviruses are also known to infect goats and sheep, but because they rarely produce clinical symptoms or elevated MSCC (Turin et al., 2005), they are not usually considered as classic small ruminant intramammary pathogens. Nevertheless, caprine lentiviruses should still be included in the general plan for controlling mastitis (Contreras et al., 2003).

Because contagious agalactia syndrome produces symptoms other than mastitis, some authors fail to consider *Mycoplasma* spp. as the etiology of sheep or goat IMI. However, the intense effects of this pathogen in reducing milk production and increasing the MSCC, means that contagious agalactia should be considered as one of the most important causes of mastitis in endemic areas, where subclinical cases are frequent. In herds clinically infected by *Mycoplasma* spp., besides significant losses due to mortality or the need to cull animals, producers cannot comply with the milk quality standards demanded by consumers, industry and public health organizations (Corrales et al., 2004).

Rather than risk a human health hazard that could be caused by some mastitis-causing bacteria, milk is generally heat treated to minimize this effect. However, in regions where cheese is made from raw milk, controlling clinical and subclinical mastitis becomes a priority. Even when using pasteurized milk, the ability of some bacteria, such as *Staphylococcus aureus*, to produce thermostable toxins, enhances the zoonotic role of these pathogens. Under European legislation, the control of *S. aureus* is mandatory, such that the marketing of sheep, goat and cow milk containing *S. aureus* is highly restricted (Directive 92/46ECC Council, 1992). Because of its zoonotic importance, preventing milk contamina-

tion by *Listeria monocytogenes* it a high priority for the industry. Although, most cases of milk-borne listeriosis are related to spoilage of the raw milk through fecal or environmental cross-contamination, a few cases of listerial mastitis have been reported in sheep. One report of clinical mastitis in a ewe caused by *L. monocytogenes* described a highly increased MSCC and persistent shedding of bacteria through milk (Winter et al., 2004).

Similarly, severe human infections attributed to the consumption of non-pasteurized cow milk were associated with mastitis caused by Streptococcus zooepidemicus (Balter et al., 2000). There have also been descriptions of mastitis due to S. zooepidemicus in goats and sheep (Las Heras et al., 2002). The identification of Nocardia spp., has also been considered important, due to their potential for causing disease in humans, and because Nocardia farcinica is known to cause mastitis in goats (Berriatua et al., 2001; Maldonado et al., 2004). Indeed, N. farcinica is a significant public health concern owing to its aggressiveness, its tendency to disseminate, its resistance to antibiotics and its laborious biochemical identification (De La Iglesia et al., 2002). These difficulties could have contributed to the increased incidence of disease caused by this microorganism in developed countries (De La Iglesia et al., 2002).

Intramammary infections caused by S. aureus warrant special attention because this bacterium is responsible for both acute clinical mastitis (gangrenous mastitis) and subclinical mastitis. S. aureus secretes several toxins contributing to the pathogenesis of mastitis and also plays a role in foodborne disease, even with pasteurized milk because of the thermostable enterotoxins. These enterotoxins are produced not only by S. aureus isolates from clinical mastitis but also by isolates from subclinical mastitis. In this sense, De Santis et al. (2005) found that the S. aureus isolates from sheep with subclinical mastitis are less enterotoxigenic (34.4%) than isolates from acute clinical mastitis (70-80%). Because of the production of these thermostable enterotoxins from S. aureus isolates, a main priority should therefore be the implementation of programs to erradicate S. aureus from dairy herds of sheep and goats.

In addition to enterotoxins produced by *S. aureus*, there is also a wide pattern of virulence factors such as the leukotoxins. These leukotoxins can selectively kill host polymorphonuclear leukocytes (PMN) and monocytes. In an investigation of the leukotoxic actions of *S. aureus* strains isolated from cows, sheep and goats with mastitis (Rainard et al., 2003) found that most isolates were leukotoxic and that strains isolated from small ruminants were more leukotoxic towards bovine PMN than *S. aureus* strains of bovine origin. However,

Table 1
Percentages of species identified from subclinical staphylococcal intramammary infection in goats

	Poutrel (1984), $n = 218$	= 218 $n = 198$ Vassiliadou al. (19)		Contreras et al. (1995), $n = 49$	Deinhofer and Pernthaner (1995), n = 303	Poutrel et al. (1996), $n = 2641$	Contreras et al. (1997a,b), $n = 130$	Leitner et al. $(2004b)$ , $n = 79$	Moroni et al $(2005)$ , $n = 1586$
SCN novobiocin-suscep	otible								
Staphylococcus spp.	11	_	3.1	4.1	_	_	_	32.9	1.6
S. aureus	N.C.	4.1	18	6.1	17.6	19.7	7.7	_	7.1
S. auricularis	_	_	_	_	_	_	_	_	
S. epidermidis	47.7	44.5	14.7	20.4	32.3	27.4	4.6	13.9	36.1
S. capitis	_	0.5	13.2	8.2	2.6	_	9.2	_	
S. caprae	19.7	_	4.5	22.5	16.5	18.8	10.8	2.5	14.6
S. chromogenes	2.8	-	_	12.3	1.3	_	23	3.8	16.6
S. haemolyticus	0.9	_	3.6	4.1	_	6.9	0.7	_	
S. hominis	_	2	6	2	1.3	_	2.4	_	
S. hyicus	1.4	11.6	12.1	8.2	_	_	_	_	
S. intermedius	_	26.3	9.9	_	_	_	_	_	
S. lugdunensis	_	-	_	_	2.3	4.3	_	_	
S. simulans	10.1	2	1.9	_	8.9	17.7	5.4	7.6	9
S. warneri	_	3	_	2	0.7	_	7.7	_	13
Novobiocin-resistant									
S. arlettae	_	_	_	2	1	_	_	_	
S. cohnii	0.4	-	3.6	2	_	_	_	_	
S. saprophyticus	_	_	7.8	_	0.3	_	_	_	
S. sciuri	3.2	0.5	_	_	0.7	_	0.7	_	
S. xylosus	2.8	1.5	_	6.1	1.6	5.2	23.8	39.2	2
S. lentus	_	_	1.6	_	12.9	_	4.6	_	

N.C., not considered.

Table 2
Percentages of species identified from subclinical staphylococcal intramammary infection in ewes

	Deinhofer (1993), $n = 72$	Marco (1994), $n = 170$	Burriel (1998), n = 38	Las Heras et al. (1999a,b), $n = 170$	Pengov (2001), n = 106	Leitner et al. $(2001)$ , $n = 107$	Ariznabarreta et al. (2002), $n = 516$	Leitner et al. (2004a), $n = 36$
SCN novobiocin-suscep	otible							
Staphylococcus spp.		1.2	31.6	2.9	26.4	26.2	2.3	
S. aureus	22.3	27.6	5.2	5.3	9.4		5.0	
S. auricularis		_	2.6				0.2	
S. capitis		_					0.4	
S. caprae		0.6		1.7	17.9		1.0	
S. chromogenes		17.6	7.9	7.1		15.9	5.2	30.6
S. epidermidis	30.6	35.9	18.4	55.9	20.7	7.5	67.4	13.9
S. haemolyticus	4.2	_	2.6	8.8		25.2	2.9	13.9
S. hominis	1.4	0.6					1.5	
S. hyicus		_	2.6		13.2	1.9	0.2	
S. intermedius		0.6				12.1	0.2	
S. lugdunensis		_				5.6	_	
S. simulans	2.8	14.1	7.9	7.1	8.5	2.8	3.7	27.8
S. warneri	6.9	0.6	7.9	3.5			_	
SCN novobiocin-resista	ant							
S. equorum	5.6							
S. lentus	13.9	0.6					1.0	
S. saprophyticus	1.4	_					_	
S. sciuri		0.6	2.6				_	
S. xylosus	11.1	_	10.5	7.6	3.8	2.8	8.9	13.9

these authors also noted that the PMN of small ruminants were more resistant to these leukotoxic effects than bovine PMN. Besides producing toxins, *S. aureus* also secrete exopolysaccharides ("slime"), which form a protective barrier that restricts the efficiency of both the host immune response and chemotherapy (Baselga et al., 1994). The best strategy for controlling intramammary infection by *S. aureus* is to remove infected animals from the herd, along with conventional precautions such as milking hygiene and dry therapy.

Coagulase-negative staphylococci (CNS) are the most prevalent pathogens causing subclinical mastitis in dairy ruminants. Although less pathogenic than S. aureus, CNS can also produce persistent subclinical mastitis, significantly increase MSCC, cause clinical mastitis (Deinhofer and Pernthaner, 1995; Contreras et al., 1997b; Ariznabarreta et al., 2002), as well as producing thermostable enterotoxins (Meyrand et al., 1999; Udo et al., 1999). Nevertheless, despite the accepted role of these bacteria as major IMI-causing pathogens in small ruminants, the pathogenicity of the different CNS species varies widely. The most commonly isolated CNS species in persistent subclinical IMI in goats and sheep are Staphylococcus epidermidis, S. caprae, S. simulans, S. chromogenes and S. xylosus (Gonzalo et al., 2002; Contreras et al., 2003; Bergonier et al., 2003). S. epidermidis and S. caprae are among the most prevalent causal microorganisms in goats and S. epidermidis and S. simulans are in ewes. The presence of different CNS species could be attributable to certain practices for controlling mastitis, such as the protocol and type of disinfectant used for teat dipping or dry-off treatments (Contreras et al., 2003). Because novobiocin-sensitive CNS seem to be the most pathogenic, we should consider including this antibiotic in the dry-off treatment procedure (Deinhofer and Pernthaner, 1995; Gonzalo et al., 2002), although maximum residue limits for sheep and goat milk have not yet been defined for this antibiotic.

Milk yield losses and increased MSCC in infected goat and sheep udders have been widely documented (Gonzalo et al., 1994, 2002; Leitner et al., 2004a,b), and it appears that sheep are more vulnerable than goats to milk yield losses due to subclinical mastitis (Silanikove et al., 2005). However, despite the high incidence of CNS linked to IMI in sheep and goats, the pathogenic mechanisms that underlie the subclinical infections remain largely unknown. Using *S. epidermidis* to induce IMI in ewes, Winter and Colditz (2002) reported that lactating udders are capable of a prominent local inflammatory response. Cytokine levels were significantly elevated soon after infection, peaking between 8 and 24 h, and increased IL-1β levels persisted for 144 h. In parallel, the

MSCC peaked at 8 h but counts returned to normal values between 48 and 144 h, despite the presence of bacteria in milk. These authors suggested a complex relationship between cytokines and the course of infection, because cytokines and PMN decreased as infection progressed. In addition, when comparing goat and sheep IMI, it seems that the sheep mammary gland is more affected by CNS (Leitner et al., 2004a,b).

### 3. Diagnostic tools for small ruminant mastitis

The gold standard for the diagnosis of IMI in dairy species is bacterial culture. Selective bacteriological testing serves to cut the cost of extensive sample collection and could help poorer areas adopt mastitis control programs. In this sense, the viability of frozen intramammary pathogens in milk is longer than the lactation period, such that frozen samples can be used in the design of goat mastitis control programs (Sanchez et al., 2003). For economic and practical reasons, usually only one milk sample is used for the diagnosis of IMI. Indeed, taking as a true positive diagnosis, the isolation of the same pathogen in consecutive samples from the same udder half, pre-milking and single sampling shows high sensitivity (96.2%) and specificity (96.1%) (Contreras et al., 1997a). Nevertheless, because the specificity and positive predictive values of this test were found to be higher for post-milking, compared to pre-milking samples, collecting a post-milking sample is recommended when only one milk sample is used for the diagnosis of goat IMI (Sanchez et al., 2004).

The most important differences between goats and sheep affecting diagnosis of mastitis are related to the MSCC. These differences are mainly due to the higher MSCC in uninfected goat halves, the higher apocrine component of goat milk secretion and the larger number of non-infectious factors that can increase the MSCC of goats compared to sheep (Paape et al., 2001). Today, most dairy laboratories use MSCC methods (fluor-optoelectronic counters) that are adequate for the apocrine pattern of milk from small ruminants, especially goats. However, given that the MSCC is an indicator of milk quality and that bonus/penalty schemes for the dairyman are based on the bulk tank MSCC, it is important that the MSCC is as accurate as possible. At present, the direct microscopic SCC method using Methylene Blue staining is the reference method recommended by the IDF (1995), but this method can overestimate the SCC of goat milk due to high concentrations of cytoplasmic particles (Paape et al., 2001).

For this reason some countries as the USA had more specific reference methods as the official standard, as

it is the Pyronin-Y Methyl Green stain (Haenlein and Hinckley, 1995; Haenlein, 2002). Similarly, the calibration of somatic cell counters for use in small ruminant's with cow milk standards has been discussed by Zeng et al. (1999), who demonstrated an overestimation of goat SCC compared to somatic cell counters calibrated with cow milk standards. The same authors also reported that a 3 day shipment of goat milk samples on ice and storing samples under refrigeration for 3 days did not affect MSCC results. Some aspects of standardization of ewe milk analyses has been recently published (Gonzalo et al., 2003, 2004a; Martinez et al., 2003). According to these authors, the reference method MDSCC (Methylene Blue stain) (IDF 148A: 1995) was a valid method in ewe milk, even though more specific stainings such as May-Grünwald-Giemsa or Pyronin Y-methyl Green increased the global accuracy for repeated SCC. Under Fossomatic method, type of cytometry (disk or flow), preservation, storage, analytical temperature and milk age showed a significant effect on SCC variation. The bromopol preserved milk stored at refrigeration temperature and analyzed at 40 °C by flow cytometry gave the optimal global accuracy over 9 days in ewe milk. In addition, SCC was lower after freezing than in refrigeration.

This effect depended specifically on type of preservation and analytical temperature of milk. The SCC of milk unpreserved or preserved with bromopol or potassium dichromate, and analyzed at  $40\,^{\circ}\text{C}$ , was not affected by freezing in the Fossomatic method. In this sense, a recent paper (Sánchez et al., 2005) demonstrated that bromopol is a suitable preservative for goat milk samples refrigerated for as long as 25 days or frozen for 25–105 days.

### 4. Control and prevention strategies

Vaccines against clinical grangrenous mastitis, that are available on the market for small ruminants, are widely used when there is a high incidence of clinical gangrenous mastitis. However, owing to the reported different effectiveness of these vaccines for dairy cows and sheep, and their inability to prevent new infections, it has been suggested that vaccines should be used in dairy herds with a high prevalence of S. aureus IMI to reduce clinical symptoms. The effectiveness of vaccination programs against mastitis caused by S. aureus has been reported for sheep but not for goats (Amorena et al., 1994; Tollersrud et al., 2002). The efficacy of a vaccine in preventing mastitis by S. aureus and S. simulans was assessed in field conditions (Marco, 1994). The results indicated a reduced prevalence of clinical mastitis but not of subclinical infections. At present, vaccination studies have failed to find this tool decisive for controlling mastitis in small ruminants, and more immunization studies are needed to improve this strategy.

To improve the health status of the herd, the whole farm has to be subjected to conditions of strict hygiene. By optimizing milking machine standards and parlor systems, the udder health of dairy sheep herds was found to improve (Gonzalo et al., 2005). Most of the routines implemented for dairy cows, including milking order, are also applicable to small ruminants, especially when the herd shows a high incidence of IMI. Because of the opportunistic nature of CNS, their prevalence increases with deficiencies in mechanical milking systems or in milking hygiene. To control CNS-induced IMI, all milking routines should be revised and milking equipment must be periodically checked to ensure correct milking variables such as vacuum level, pulsation rate and ratio, vacuum reserve per milking unit, etc. Similarly, adequate quality control of the water used to clean the milking equipment is needed to avoid infection outbreaks, as has been reported for P. aeruginosa (Las Heras et al., 1999b).

Teat dipping has been demonstrated to be highly effective at preventing new intramammary infections in cows from different pathogens, especially CNS (Hogan et al., 1987). In small ruminants, post-milking teat dipping has been used mainly in highly infected herds (Paape et al., 2001; Bergonier and Berthelot, 2003; Contreras et al., 2003), and it has been revealed as a very effective method to prevent new intramammary infections. However, the quality control of the teat dip disinfectant is very important, because some sporadic outbreaks have been related to an inadequate disinfectant acting as an infection source, as reported for *S. marcescens* causing mastitis in sheep when using a quaternary ammonium based teat dip (Tzora and Fthenakis, 1999).

Conventional teat dipping solutions are either iodine or chlorine based, which are not suitable for organic farming, and studies are underway on the efficiency of new disinfectants. In one study, a dodecyl benzene sulfonic acid spray failed to maintain and/or restore the udder health of a sheep herd subclinically infected by CNS (Klinglmair et al., 2005). The efficacy of teat dip disinfectants suitable for organic farming is an interesting area of research that should be approached in the future for dairy small ruminants. In addition, the role of the disinfectant used on public health issues has been pointed out by Bjorland et al. (2005), who demonstrated the widespread distribution of disinfectant resistance genes among Staphylococci in both bovine and caprine milk. So, the control of the disinfectant used should be implemented in the future, as it is for antibiotics, to avoid development of widespread numbers of multi-resistant

strains of bacteria, which could be a potential hazard for humans.

Controlling the epidemiological situation of S. aureus-provoked IMI in herds of nursed lactating animals is difficult, because the lambs or kids of infected mothers will transmit the infection to the rest of the lactating females, when trying to supplement their own mothers' milk. Also, in sheep with IMI due to M. haemolytica, suckling lambs are the main source of infection as they spread the infection to their mothers. Weaning leads to a drop in the incidence of M. haemolytica mastitis. Fortunately, use of pasteurized colostrum is on the increase in modern dairies for production and health reasons, such as combating lentivirus infection (Contreras et al., 2004). Because this procedure allows removal of the newborns at parturition from their mothers with the feeding colostrum and milk free of pathogens, it is improving the health status of both the kids or lambs and the mothers' udders.

Antibiotic dry-off therapy was found to significantly reduce the incidence of IMI in dairy ewes and goats (McDougal and Anniss, 2005; Gonzalo et al., 2004b). Although some authors (Poutrel et al., 1997) pointed out, that generalized intramammary antibiotic should be applied in high prevalence conditions, selective rather than generalized dry-off antibiotic treatment seems to be preferable based on the following findings:

- The spontaneous cure rate at parturition, which can be especially high for small ruminants, is 20–60% (Paape et al., 2001; Contreras et al., 2003; Bergonier and Berthelot, 2003). In a study in which the incidence of IMI during the postpartum period was compared in goats and sheep, spontaneous cure rates were significantly higher in sheep (McDougall et al., 2002).
- 2. Excellent improvement of udder health and bulk tank MSCC, when adequate programs to control mastitis are correctly implemented (Contreras et al., 2003; Gonzalo et al., 2005). In modern milking farming, the proper maintenance of the milking machine and the milking parameters and the correct milking routines are essential to minimize risk factors for IMI and when they are well performed, udder health increases comparing with hand milking.
- 3. The implementation of good dairy farming practices. Consumers want to verify the quality of milk products through use of quality standards. Mastitis degrades milk quality and diminishes the ability of the dairy industry to compete in international markets. To help dairy technicians and dairymen optimize the mammary health of the flocks and to improve milk quality,

- some cooperatives or association of small ruminant dairy farmers have started to develop programs of farm audits and the use of farm guidelines for mastitis control (Gonzalo et al., 2004c). However, programs implemented on farms for dairy cows cannot be directly applied to farms for small dairy ruminants. Differences in size of the herds, marginality of some areas for raising small ruminants, the low income of the producers, the particular shepherding systems, the difficulties in keeping routine individual records, and other particularities make small ruminant species very different from dairy cows and require the design of specific strategies for control of milk quality. Within the next few years a priority will be the defining of specific standards of milk hygiene and quality of small ruminant milk, and implementation of good management guidelines for these species.
- 4. Antibiotic treatment should require veterinary surveillance to ensure adequate and hygienic administration. Some massive outbreaks of mastitis have been attributed to an iatrogenic origin through syringe contamination by *P. aeruginosa* or *A. fumigatus* (Las Heras et al., 1999b; Bergonier et al., 2003; Contreras et al., 2003; Gonzalo et al., 2004b). A sporadic outbreak caused by *B. cepacia* was also associated with contamination during antibiotic dry-off treatment (Berriatua et al., 2001).
- 5. Overuse of antibiotics increases the risk of antibiotic resistance and has become a public health problem. The detection of *S. aureus* strains in sheep resistant to aminoglycoside antibiotics should be considered a public health concern, given the similar resistance mechanism to strains isolated in humans (Goni et al., 2004).
- 6. Few drugs are specifically licensed for use in small ruminants, particularly goats. The use in small ruminants of antibiotics or other drugs registered for cows, or even the use in goats of products registered for sheep, carries a high risk because the safety and efficacy of these products in each species are largely unknown (Mavrogianni et al., 2004).
- 7. The absence of antibiotic residues in milk from cows and other species is mandatory in the European Union, although it seems that positive results are higher in milk from small ruminants than in cow's milk (Yamaki et al., 2004). Despite the availability of registered non-specific methods of residue detection for the milk of small ruminants, Contreras et al. (1997b) demonstrated the high selectivity of several antibiotic residue kits registered for cow's milk towards goat's milk. However, because the techniques

routinely used for identifying antibiotic residues are unable to detect all positive cases, antibiotic detection methods need to be standardized for sheep and goat milk (Yamaki et al., 2004; Montero et al., 2005).

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